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A mechanism-based cancer risk assessment for 1,4-dichlorobenzene

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Abstract

1,4-Dichlorobenzene (DCB) induced liver cancer in male and female B6C3F$_1$ mice in a gavage bioassay and in male and female BDF$_1$ mice in an inhalation bioassay. The weight of the evidence convincingly indicates that the mouse liver tumors induced by 1,4-DCB were via a nongenotoxic-mitogenic/promotional mode of action by forcing the growth of spontaneous precancerous lesions. Doses insufficient to exhibit mitogenic or promotional activity would not be expected to increase the risk of cancer. Benchmark dose modeling of the tumor response was conduced for the combined inhalation and oral gavage bioassay data sets based on an absorbed dose basis to establish the dose or airborne concentration corresponding to 1% extra risk. Assuming that as a point of departure and dividing by an uncertainty factor of 300, yielded a value of 0.1 ppm, representing a rational estimate of an airborne concentration for the human population below which there is unlikely to be any increased risk of cancer during a lifetime. In contrast, the default model that assumes a genotoxic mode of action estimates a one in one-million increased lifetime risk of cancer at an airborne concentration of 0.00004 ppm, some 2500-fold lower than the mechanism-based model and 1,875,000-fold lower than the no observed effect concentration for induced cancer of 75 ppm in the inhalation bioassay.

Keywords: 1,4-Dichlorobenzene; Cancer risk assessment; Nongenotoxic-mitogenic carcinogens

1. Introduction

1,4-Dichlorobenzene (1,4-DCB) is utilized in industrial processes as well as consumer products such as mothballs and deodorizing cakes in urinals resulting in a low level of environmental airborne exposure to 1,4-DCB for some segments of the population (Commonwealth of Australia, 2000; European Union, 2004; U.S. EPA, 2006b). In a long-term gavage study, 1,4-DCB induced cancer in the livers of male and female B6C3F$_1$ mice and in the kidneys of F344 male rats (NTP, 1987). In a long-term inhalation study, 1,4-DCB induced cancer in the livers of male and female BDF$_1$ mice, while no increase in tumors was observed in exposed F344 rats (Aiso et al., 2005). A critical issue with any carcinogen, and in particular with a high profile chemical such as 1,4-DCB, is developing an understanding of the mechanism of action so that the most scientifically valid risk assessment can be applied in formulating health protective exposure guidelines. Fortunately, a great deal of research has been conducted on the mechanisms by which 1,4-DCB produces cancer. The purpose of this paper is to define a mechanism of action based cancer risk assessment for 1,4-DCB. The following outlines the weight of evidence for the mechanism of action for 1,4-DCB induced tumors and provides an appropriate quantitative cancer risk assessment.

2. Use of mode of action as the basis for relevant cancer risk assessments

Carcinogenesis is a complex, multi-stage process involving the sequential mutation of growth control genes and the clonal expansion and progression of the resulting

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precancerous and cancerous cells to fully a malignant tumor. Different chemicals may exhibit carcinogenic activity by affecting one or more of the many events that can lead to tumor formation. Several processes have been well established as important in driving tumor formation by various chemical agents (Butterworth, 2006). Genotoxic mutagenic or clastogenic carcinogens or metabolites can induce mutations in growth control genes directly via a DNA reactive pathway. Continual administration of cytotoxic carcinogens at cytolethal levels can result in a range of DNA damaging side effects and promotional activity at the site of injury, such as inflammation, oxidative stress, regenerative cell proliferation, and release of growth factors, all of which can participate in the carcinogenic process. Receptor-mediated mitogenic carcinogens when administered on a continual daily basis stimulate cellular growth and increased organ weight. These mitogenic chemicals often exhibit promoting activity by providing a selective growth advantage to chemically- or spontaneously-initiated precancerous and cancerous cells.

The concept of mode of action is a useful strategy to focus on the rate limiting events that can be used as the bases for choosing appropriate cancer risk models. Various similar strategies have been proposed for defining mode of action (Ashby and Tennant, 1991; Butterworth, 2006; Butterworth et al., 1995; Butterworth and Bogdanffy, 1999; Dearfield et al., 1991; Holsapple et al., 2006; IPCS, 1999; Meek et al., 2003;Tennant, 1993; U.S. EPA, 2005). Mode of action can be defined as a fundamental obligatory, or rate limiting step in the induction of toxicity or cancer and allows carcinogens to be placed in categories that best describe the primary biological activity driving tumor formation. Conditions where this critical event in tumor formation does not occur, such as with lower doses or in species-specific responses, would result in no increase in the cancer risk. For example, a nongenotoxic-cytotoxic mode of action may establish induced cytolethality as the key primary event, while acknowledging that numerous initiation and promotional activities may result from that cytolethality. Thus, cytolethality is the rate limiting step because absent that, none of the subsequent events would follow. This knowledge also facilitates the approach that a more readily quantified noncancer endpoint can be used in the risk assessment as a surrogate for carcinogenic activity when actual tumor data are not available (Butterworth and Bogdanffy, 1999). Important categories of modes of action include genotoxic, nongenotoxic-cytotoxic, and nongenotoxic-mitogenic.

A two-part framework has been proposed for assigning a mode of action (Butterworth, 2006). First, a weight of evidence approach is used to assess mutagenic potential based on results of genetic toxicology test systems. Second, a descriptor is assigned to classify the degree to which mutagenic activity likely played a role in the mode of action of tumor formation. This option provides a more realistic way of describing the mode of action instead of being bound by the strict genotoxic vs. nongenotoxic choices.

3. Target tissues for cancer risk assessment for 1,4-DCB

3.1. Gavage bioassay

A bioassay was conducted by the National Toxicology Program (NTP) with 1,4-DCB administered in corn oil by gavage to male and female $B6C3F_1$ mice and female F344 rats at 300 and 600 mg/kg/day, 5 days per week (NTP, 1987). Male F344 rats were given the same regimen, but the doses were 150 and 300 mg/kg/day.

3.1.1. Response in mice

Significant increases in the frequency of hepatocellular carcinomas and adenomas were seen in both sexes of mice at the 600 mg/kg/day dose. An increase in hepatocellular adenomas was seen in the male mice at the 300 mg/kg/day dose.

3.1.2. Response in rats

No significant increases in liver cancer were seen in F344 rats in any of the dose groups. 1,4-DCB produced a dose-related increase in the incidence of renal tubular cell adenocarcinomas in the male rats. The male rat kidney tumors were judged to have been produced via the nongenotoxic-cytotoxic alpha-2u-globulin pathway (Bomhard et al., 1988; Borghoff et al., 1990; Charbonneau et al., 1989; NTP, 1987). That pathway is considered to be specific to the male rat with no counterpart for human beings. Thus, the male rat kidney tumors are not relevant for human cancer risk assessments (U.S. EPA, 2005, 2006b).

3.1.3. Limitations of the gavage bioassay

Concerns with the 1987 NTP study are that the 1,4-DCB was administered by bolus gavage and only two dose groups were used. The extremely high dose-rate associated with gavage can substantially alter the physiology and toxicokinetic profile within the animal as well as overwhelm defense mechanisms that would be protective were the dose spread out, such as by inhalation or in the drinking water (Larson et al., 1994). Further, both of the doses used in the mice produced not only a mitogenic enlargement of the liver, but the final sacrifice also revealed histopathological changes including hepatocellular degeneration and focal necrosis (Table 1). One should not extrapolate the liver tumor response in mice at doses where mitogenic activity, cytolethality, and regenerative cell proliferation were playing an active role in tumor induction to normal environmental exposures where such factors are not in play (Butterworth, 2006). In summary, the cancer risk assessment from the gavage bioassay should be based in the incidence of male and female mouse liver tumors. Those data are, however, limited in that only two doses were examined and consideration must be given to the mitogenic as well as

Table 1
Incidence of liver tumors in male and female mice from NTP (1987) bioassay

Table 19 from NTP (1987). Number of mice with liver lesions in the two-year gavage studies of 1,4-dichlorobenzene						
Lesion	Male			Female		
	Vehicle control	300 (mg/kg)	600 (mg/kg)	Vehicle control	300 (mg/kg)	600 (mg/kg)
Number of mice examined	50	49	50	50	48	50
Hepatocellular degeneration	0	36	39	0	8	36
Cell size alteration	0	38	40	0	4	27
Focal necrosis	1	35	37	1	4	30
Hepatocellular adenoma	5	13	16	10	6	21
Hepatocellular carcinoma	14	11	32	5	5	19
Hepatoblastoma	0	0	4[a]	0	0	0

[a] All hepatoblastomas were observed in mice that had hepatocellular carcinomas.

cytotoxic activity that was involved in driving tumor formation.

3.2. Inhalation bioassay

The Japan Bioassay Research Center (JBRC) conducted a two year chronic bioassay with both sexes of BDF_1 mice and F344 rats exposed to airborne target concentrations of 1,4-DCB of 20, 75, or 300 ppm, 6 h/day, 5 days/week (Aiso et al., 2005). One strength of the inhalation bioassay is that three exposure groups were used, thus providing a better characterization of the dose-response curve (Aiso et al., 2005). Further, exposures were below levels that produced overt hepatocellular cytolethality. Thus, the tumor induction responses in this study are more applicable in estimating inhalation exposure risks than are the responses from the 1987 NTP gavage study.

3.2.1. Response in mice

An increase in hepatocellular carcinomas were seen in both sexes of mice at 300 ppm, but not in either the 20 or 75 ppm exposure groups (Table 2). An increase in hepatocellular adenomas was also seen in the female mice at 300 ppm.

3.2.2. Response in rats

No induction of cancer was observed in either sex of the F344 rats in any of the exposure groups (Table 3).

3.2.3. Bronchoalveolar tumors are not increased

Upon completion of the inhalation bioassay with 1,4-DCB, the JBRC issued for informational purposes a preliminary and non-peer reviewed summary of some of the results of the 1,4-DCB inhalation bioassay (JBRC, 1995). Reference is sometimes made to this summary even though it has never been published. It is important to note that that this summary does not contain the final conclusions based on a critical review of the data by the study authors as given in the peer reviewed publication (Aiso et al., 2005).

The preliminary JBRC summary of the cancer inhalation bioassay noted no significant increase in the incidence of bronchiolar-alveolar adenomas or bronchiolar-alveolar carcinomas in either sex of mice (JBRC, 1995). When the adenoma and carcinoma incidences were combined for the female mice, however, a positive trend was judged as significant by the Peto test, even though no dose–response was evident and the incidences were within the historical control values for that testing laboratory. Importantly, when the Aiso et al. study was published, bronchoalveolar cancer was not listed as one of the tumor types induced by 1,4-DCB (Aiso et al., 2005). The study authors clearly and specifically discount the lung tumors as not being related to chemical exposure, concluding that the observed incidences did not exceed the historical control data range for that laboratory (Aiso et al., 2005). Since there was no induced increase in the incidence of bronchoalveolar tumors, they are not relevant for a quantitative cancer risk assessment.

3.3. Mouse liver tumors as the basis for cancer risk assessments

In summary, the only relevant tumor type of concern and upon which cancer risk assessments should be based for 1,4-DCB are the liver tumors induced in the gavage

Table 2
Inhalation cancer bioassay liver weights and incidence of liver tumors at two years in mice (Aiso et al., 2005)

1,4-DCB (ppm)	Male BDF_1 mice				Female BDF_1 mice			
	0	20	75	300	0	20	75	300
Liver wt. (% b.w.)	4.21	5.06	4.87	8.61*	5.01	5.62	5.02	17.95*
Fold increase	1.0	1.2	1.2	2.0	1.0	1.1	1.0	3.6
Hepatocellular carcinoma (%)	24	34	32	78*	4	8	4	82*
Fold increase	1.0	1.4	1.3	3.3	1.0	2.0	1.0	20.5

* Statistically significant increase.

Table 3
Inhalation cancer bioassay liver weights and incidence of liver tumors at two years in rats (Aiso et al., 2005)

1,4-DCB (ppm)	Male F344 rats				Female F344 rats			
	0	20	75	300	0	20	75	300
Liver wt. (% b.w.)	3.33	3.58	3.63	3.95	2.64	2.66	2.77	3.16
Fold increase	1.0	1.1	1.1	1.2	1.0	1.0	1.0	1.2
Hepatocellular carcinoma (%)	No induced liver cancer				No induced liver cancer			

bioassay in male and female $B6C3F_1$ mice and in the inhalation bioassay in male and female BDF_1 mice (NTP, 1987; Aiso et al., 2005).

4. Evaluation of genotoxic potential

4.1. Importance of a weight of the evidence approach

The first step in the process of defining a mode of action for a carcinogen is to evaluate the direct acting, DNA reactive, mutagenic or genotoxic potential of the chemical or its metabolites (Butterworth, 2006; U.S. EPA, 2005). Making that classification can be more difficult than it might appear. Many proposed genotoxicity tests have had only minimal validation with sets of known mutagens and known nonmutagens. Assays have often been engineered to be highly sensitive to mutagenic activity, and, consequently, exhibit varying degrees of false positive responses. Chemicals often exhibit activity in bacterial or cell culture models that do not translate into measurable analogous activity in the whole animal where processes of uptake, distribution, metabolism, detoxification, DNA repair, and excretion come into play. It is rare for any chemical that has undergone substantial testing not to have one or two reported positive mutagenicity tests, even for substances which are clearly nongenotoxic (Waters et al., 1991; Kirkland et al., 2005). The body of genetic toxicology data requires extensive thoughtful review by individuals who know the strengths and weaknesses of the assays. In cases such as 1,4-DCB where numerous genetic toxicology assays have been run, a weight of the evidence approach is used to critically review the data, giving more weight to studies that have been well conducted using validated test systems.

4.2. The weight of the evidence indicates that 1,4-DCB is nongenotoxic

1,4-DCB has been subjected to an exceptionally large number of genotoxicity tests. Discussion of this very large literature and data set is beyond the scope of this paper. The overall weight of the evidence for the genotoxic potential of 1,4-DCB is best found in the conclusions from several expert groups that have reviewed the database. An exceptionally well researched critical review of the genetic toxicology, cell proliferation, and cancer profiles of 1,4-DCB was done by the Beraterkreis Toxikologie, a German group that has deliberated the status of 1,4-DCB (Beraterkreis Toxikologie, 2003). The conclusion of that body is: "Overall, the data do not support a classification of p-DCB as a mutagen." The European Union (2004) (Risk Assessment Report on 1,4-DCB) concludes: "The overall weight of evidence from the most reliable studies indicates that 1,4-DCB does not have any significant genotoxic potential." The NICNAS review of 1,4-DCB conducted by the Commonwealth of Australia (2000) reaches the same conclusion. Taken together, the weight of the evidence strongly indicates that 1,4-DCB is unlikely to induce cancer via a direct acting, mutagenic or genotoxic mode of action.

The general pattern of data indicate that 1,4-DCB is negative in vitro and in vivo in a battery of standard, proven genotoxicity assays. A sentence from the U.S. EPA IRIS review provides an indication of some of the kinds of assays that were negative with 1,4-DCB: "Negative results were reported in the vast majority of a variety of assays, including gene mutation in *Salmonella typhimurium* and mouse lymphoma cells in vitro; DNA damage in rat and human hepatocytes in vitro; unscheduled DNA synthesis in mouse hepatocytes and rat kidney cells in vivo, sister chromatid exchange in CHO cells in vitro; mouse bone marrow cells and erythrocytes in vivo; chromosomal aberrations in rat bone marrow cells in vivo; and dominant lethal mutations in mice (U.S. EPA, 2006b)." The exceptions to the negative responses generally fall into the categories of (1) results that are not reproducible; (2) tests that are more unconventional and less well validated such as the micronucleus test in rat kidney (validation means that test performance has been evaluated with a large set of known mutagens and known nonmutagens); and (3) assays that are prone to false positives due to toxicity, such as the alkaline elution assay, the comet assay, and the SCE assay. In our opinion the weight of the evidence is convincing that 1,4-DCB does not induce cancer via a DNA reactive, mutagenic, or genotoxic mechanism.

5. Mitogenic/promotional mode of action

The characteristics of the mitogenic/promotional mode of action for a liver carcinogen are the stimulation of growth of the liver as well as stimulation of growth of precancerous lesions, but only so long as the compound is continually administered. A substantial scientific literature is available describing the mitogenic/promotional mode of action (Cattley et al., 1991; Eldridge et al., 1992; Marsman and Popp, 1994; Schulte-Hermann and Parzefall, 1981; Schulte-Hermann et al., 1981, 1982, 1983; Watanabe and

Williams, 1978). Dr. Rolf Schulte-Hermann and coworkers have published a particularly clear overview of the nature of such chemicals (Schulte-Hermann et al., 1983).

A number of different liver carcinogens fall in the class of nongenotoxic-mitogenic carcinogens including phenobarbital, hypolipidemic drugs, some sex steroids, and chlorinated hydrocarbons including DDT, hexachlorocyclohexane and 1,4-DCB (Schulte-Hermann et al., 1983). The stimulation of liver growth and a sustained increase in liver weight, so long as the chemical is continually administered on a daily basis, is one effect common to all of the mitogenic liver carcinogens. Mitogenic activity in the mouse liver was clearly seen early and late in both the gavage and inhalation bioassays with 1,4-DCB (NTP, 1987; Aiso et al., 2005; Eldridge et al., 1992). There was no regenerative cell proliferation in the inhalation study or early in the gavage study because no liver cell death or necrosis was occurring. In the case of induced mitogenic activity, the cell turnover rate may actually return to normal levels, but the livers remain enlarged so long as the 1,4-DCB is continually administered. However, in the gavage bioassay, doses were so high that liver necrosis and cytolethality (and very likely regenerative cell proliferation) were also seen at the final sacrifice (NTP, 1987).

The driving force for tumor formation appears to be the promotion of liver tumors from spontaneously induced precancerous, or induced, liver cells. These mitogens provide a selective growth advantage to preneoplastic lesions as evidenced by dramatically increased levels of cells in S-phase within preneoplastic lesions (Schulte-Hermann et al., 1983; Marsman and Popp, 1994). If animals are initiated with a liver mutagen, promotion with mitogenic agents can produce precancerous liver lesions in just a few weeks. In contrast, these precancerous lesions will appear in initiated animals not treated with mitogens only after about 6 months and in some cases only after a year or two (Schulte-Hermann et al., 1983). Selective growth may also arise from inhibiting the growth of normal cells, and/or inhibiting the rate of apoptosis (programmed cell death) of precancerous cells (Schulte-Hermann et al., 1983).

If such precancerous lesions were spontaneous in nature, rather than chemically induced, one would predict that they would increase in number with the age of the animal without chemical treatment. That is, in fact, the case. Twenty-two weeks of dietary administration of the nongenotoxic-mitogenic promoter Wy-14,643 produced a 5- to 7-fold higher yield of liver tumors in rats starting at age 15 months vs. in rats starting at age 2 months (Cattley et al., 1991).

6. Evidence that 1,4-DCB is acting via a mitogenic mode of action

Key experimental results that indicate that 1,4-DCB is driving tumor induction via a mitogenic mode of action are summarized as follows.

1. A 90 day gavage study was conducted in male and female $B6C3F_1$ mice under conditions of the cancer bioassay (Eldridge et al., 1992). In that study 1,4-DCB given daily induced an increase in liver weight in the male and female $B6C3F_1$ mice. When the compound was withdrawn, the livers returned to normal size, as is typical for mitogenic agents.
2. In the Eldridge et al. (1992) study, a dramatic increase in the percentage of cells in S-phase (labeling index) was observed, indicating that the liver cells were not just increasing in size, but that the actual number of liver cells was increasing.
3. In the Eldridge et al. (1992) study, histopathological evaluation revealed no evidence of hepatocellular necrosis and no elevations in liver-associated plasma enzymes were seen. Thus, in that study the cell proliferation was mitogenic in nature rather than regenerative.
4. The dose dependent increase in liver weights in the Eldridge et al. (1992) study was similar to the dose dependent increase in liver weights described in the gavage cancer bioassay (NTP, 1987). As expected, this parameter was seen in parallel to liver tumor induction.
5. Similarly, increases in liver/body weight ratios were seen in the Aiso et al. (2005) inhalation bioassay that were directly proportional to the incidence of liver tumors in the male and female BDF_1 mice.
6. The dramatic nonlinearity and correlation between increased liver weight and eventual tumor formation are clearly evident in the inhalation study (Aiso et al., 2005). In that study, liver tumors were induced only at the highest airborne concentration of 300 ppm that also produced dramatic increases in liver size. The next lower concentration of 75 ppm represented a no observed adverse effect level (NOAEL) for the induction of increased liver weight, the induction of altered cell foci, as well as the induction of liver tumors.
7. In no case with 1,4-DCB have liver tumors been induced without preceding large increases in liver/body weight ratios. All of the above observations constitute a cohesive and classical pattern of activity observed for chemicals that have been characterized as acting via a nongenotoxic-mitogenic/promotional mode of action (Schulte-Hermann et al., 1983).

7. Lack of rat liver tumors is not evidence against a mitogenic mode of action

Substantial species-to-species, strain-to-strain, and organ-to-organ differences in susceptibility are common for any given carcinogen. Rats are less prone to induced or spontaneous liver tumors than mice. For example, the frequency of the liver carcinomas in the control male F344 rats and male BDF_1 mice in the inhalation study, and the male $B6C3F_1$ mice in the gavage study were 0, 24, and 30%, respectively (Tables 3 and 4). $B6C3F_1$ mice are genetically predisposed to liver cancer (Drinkwater,

Table 4
Administered and absorbed doses and liver tumor responses (liver carcinomas and adenomas and carcinomas combined) for male $B6C3F_1$ mice (NTP, 1987) administered 1,4-DCB via gavage and male BDF_1 mice (Aiso et al., 2005) exposed to 1,4-DCB via inhalation

Administered dose		Absorbed dose (mg/kg-d)[a]	Total # male mice in exposure group (survival adjusted)	Number of mice with tumors	
Inhalation (ppm)	Oral (mg/kg-d)			Liver carcinomas	Liver adenomas and carcinomas combined
0		0	49	12	20
	0	0	46	14	17
20		22	49	17	21
75		81	50	16	18
	300	193	40	11	22
300		324	49	38	41
	600	386	42	32	40

[a] Absorbed doses for the inhalation bioassay were calculated assuming 60% absorption (per Aiso et al., 2005), an inhalation rate of 0.028 m^3/h, and a bodyweight of 40 g. Absorbed doses from gavage exposure were calculated assuming 90% absorption (U.S. EPA, 2006a, 2006b). Absorbed doses from both routes of exposure were adjusted to account for dosing 5 days per week (5/7).

1994); the historical control rate of liver tumors in this strain in the NTP program at the time of the gavage bioassay was 33% (NTP, 1987). In contrast, the incidence rate for liver and intrahepatic bile duct cancer in white males in the United States is only 3.7 per 100,000 (NCI, 2007). The dramatically lower incidence of liver cancer in humans compared to rodents is evidence of decreased susceptibility for this target organ in humans. The mitogen 1,4-DCB induced a mild mitogenic response in the rat liver, but no liver tumors were induced (Table 3). That observation is not evidence against a mitogenic mode of action in the mouse liver. The eventual tumor response in a tissue is dependent on many factors. Mitogenic activity is necessary, but not always sufficient, for 1,4-DCB to produce cancer. This is analogous to the situation with genotoxic agents. An alkylating mutagen will produce DNA adducts in many different tissues. Yet, eventual tumors appear only in select target organs. The lack of tumors in the presence of DNA adducts is not evidence against a mutagenic mode of action in those sites where cancer is observed.

8. Cancer risk models for mitogenic carcinogens

To be effective in inducing tumors, mitogenic carcinogens must produce conditions selective to the growth of precancerous cells for a period long enough to enhance lesion development. Doses or exposure periods insufficient to produce those effects would not be expected to induce cancer. If treatment with a mitogen is stopped, the liver rapidly returns to its normal size (Schulte-Hermann et al., 1983). A most important observation is that precancerous adenomas induced in the liver of F-344 rats by continual treatment with the mitogen Wy-14,643, regressed almost completely when the compound was withdrawn and the animals were held without further treatment (Marsman and Popp, 1994).

A variety of short-term parameters were examined in order to identify potential markers for nonmutagens that would be predictive of carcinogenic potential (Elcombe et al., 2002). In the mouse liver, the only parameter that correlated with tumor potential with a high degree of accuracy was with induced increases in the relative liver weight. One of the chemicals examined in that study was 1,4-DCB.

Results from a rat liver initiation-promotion study are consistent with the threshold nature of the carcinogenic potential of 1,4-DCB (Gustafson et al., 1998). Male F344 rats were given a single gavage injection of 200 mg/kg of the potent mutagen dimethylnitrosamine (DMN). That represents an overwhelming mutagenic assault on the liver cells and has been shown to create a large population of initiated or preneoplastic cells. Following such a genotoxic insult, promotion by adequate doses of an effective liver promoter would be expected to yield preneoplastic foci in the liver. Rats were promoted with 14.7 or 58.8 mg/kg-day of 1,4-DCB by gavage for 6 weeks. It is critical to note that while this represents a significant exposure, neither of those doses would have been high enough to induce liver cancer in either F344 rats or in $B6C3F_1$ mice (NTP, 1987). Thus, if tumor induction by 1,4-DCB by the non-genotoxic-mitogenic/promotional mode of action requires a threshold dose, one would predict no preneoplastic foci or tumor induction by the doses used, even with an abundance of initiated hepatocytes. That was the case; no preneoplastic foci were produced. The inability of lower doses of 1,4-DCB to promote the development of tumors from liver cells, even in the extreme case of initiation by DMN, is consistent with the threshold nature of the promoting potential of 1,4-DCB.

It is instructive to examine the shape of the dose–response curves for the induction of liver cancer in the Aiso et al. (2005) inhalation bioassay with 1,4-DCB (Table 2). For the sake of this comparison, the incidences of hepatocellular carcinomas were used, but adenomas follow the same pattern. A key observation is the correlation between the fold increase in liver weight and the eventual induction of liver tumors in the mice. Note the significant nonlinearity in the data with a dramatic jump in liver weight and liver cancer in going from 75 to 300 ppm. It is important to appreciate, also, that these liver weights had been elevated for two years, as an indication of the extent of the

promoting potential that had been exerted on these livers. It is also interesting to note the minimal response in increased liver weight in the rats and the corresponding lack of liver tumor induction (Table 3).

9. Mechanism-based cancer risk assessment

The strongest scientific evidence indicates that 1,4-DCB cancer risk assessments should be based on a nongenotoxic-mitogenic mode of action. The primary driving force exerted by 1,4-DCB in inducing cancer in the rodent bioassays is by forcing the growth of spontaneous precancerous lesions. This mode of action would predict a nonlinear dose–response relationship with no excess tumors at exposure regimens or doses that are insufficient to exhibit mitogenic or promotional activity and sustained increases in liver weight. No tumorigenesis would be expected to occur at doses below those that produce changes in liver weight, signaling biologically relevant mitogenic action. The parallel and one-step pattern of both tumor and mitogenic activity in the mouse liver (Aiso et al., 2005) experimentally confirm this principle. Fortunately, in the case of 1,4-DCB, the threshold nature of the tumor response is clear in the measurable range of the data. Uncertainty factors can be applied to the NOAEL or to the point of departure for the tumor dose–response curve, as determined by benchmark dose analysis, to arrive at an estimated safe exposure level.

10. The gavage bioassay is inadequate as the basis for derivation of an oral slope factor

10.1. Insufficient dose groups

The current cancer guidelines (U.S. EPA, 2005) recommend use of at least three dose groups in chronic bioassays in order to provide an indication of the shape of the dose–response curve. The limited number of dose groups and the high incidence of tumors in both exposed groups in the oral gavage bioassay (NTP, 1987) resulted in an inability to identify a benchmark dose in the observed dose range associated with any response lower than 50% extra risk. This benchmark response (BMR) is entirely too high to provide any insight or confidence in the shape of the dose–response curve at low oral doses. By itself, this dataset is clearly inadequate as the basis for quantitative low-dose extrapolations.

10.2. Substantial liver toxicity

U.S. EPA cancer guidelines caution against using tumor data for quantitative, low-dose extrapolation when clear evidence of cytotoxicity is present (U.S. EPA, 2005, pp. 2–17 and 2–18):

> "Overt toxicity or qualitatively altered toxicokinetics due to excessively high doses may result in tumor effects that are secondary to the toxicity rather than directly attributable to the agent..."
>
> "Other signs of treatment-related toxicity associated with an excessive high dose may include (a) significant reduction of body weight gain (e.g., greater than 10%), (b) significant increases in abnormal behavioral and clinical signs, (c) significant changes in hematology or clinical chemistry, (d) saturation of absorption and detoxification mechanisms, or (e) *marked changes in organ weight, morphology, and histopathology...*" [emphasis added]
>
> "Studies that show tumor effects only at excessive doses may be compromised and may or may not carry weight, depending on the interpretation in the context of other study results and other lines of evidence. *Results of such studies, however, are generally not considered suitable for dose–response extrapolation if it is determined that the mode(s) of action underlying the tumorigenic responses at high doses is not operative at lower doses*". [emphasis added]

In the case of the NTP (1987) gavage bioassay, only two doses were used, and both doses produced "marked changes in organ weight, morphology, and histopathology." The doses used were so high that not only was mitogenic liver enlargement induced, but beyond that substantial and pervasive hepatotoxicity in mice occurred at both tested doses. The additional hepatolethality may have been induced in the gavage study because the oral route of exposure provides a more direct transfer of xenobiotics to the liver through the portal vein. Table 1 is a reproduction of Table 19 from the NTP bioassay report. Note that in male mice at the lower tested dose, hepatocellular degeneration, cell size alterations, and focal necrosis were observed in more than two-thirds of the tested animals. Unfortunately, the bioassay report does not provide animal-by-animal tabulation of the nonneoplastic lesions, so comparison of the occurrence of toxicity with the occurrence of tumors on an animal-specific basis is not possible.

At the bioassay doses, induced hepatocyte proliferation reflects the mitogenic response (Eldridge et al., 1992), while necrosis is indicative of cytolethality, neither of which would be expected to be operative at lower doses that do not affect the liver weight. In addition, at the doses used, substantial induction of CYP2B protein and enzyme activity was likely (Lake et al., 1997), potentially altering the metabolism and toxicokinetics of DCB. Thus, taken alone, the NTP (1987) gavage bioassay is inadequate to support quantitative low-dose extrapolation of a cancer response.

11. The inhalation bioassay supports a threshold response

The inhalation bioassay conducted by Aiso et al. (2005) found a clear NOAEL for liver tumors in both male and female BDF_1 mice. The two lowest airborne exposure concentrations of 20 and 75 ppm produced no excess incidence of liver adenomas/carcinomas or increase in liver weight

(Table 2). Induction of increased liver weight and corresponding liver tumors were seen only at the highest inhalation exposure of 300 ppm. This is consistent with other findings that indicate that no tumorigenic response occurs until doses exceed those required to produce a mitogenic response sufficient to produce liver weight changes (Elcombe et al., 2002). The clear presence of a NOEL in this bioassay (for both tumors and liver weight changes) in the measurable range of the dose–response curve is strongly supportive of a nonlinear dose–response for DCB-induced liver tumors.

12. Integrated assessment of mouse liver tumors in the oral and inhalation bioassays: Use of absorbed dose

As noted by Aiso et al. (2005), the liver tumor responses observed in NTP (1987) oral gavage bioassay are consistent with those observed in Aiso et al. (2005) inhalation bioassay when evaluated on an absorbed daily dose basis. While this is a rational scientific approach to equating the internal doses, it is recognized that there are potential differences in underlying toxicokinetic and toxicodynamic parameters, particularly at the high gavage doses. Table 4 presents the estimated absorbed doses and tumor responses. The legend for Table 4 gives the assumptions used for calculating absorbed doses for inhalation and oral gavage exposures. The response rates from the two bioassays on an absorbed dose basis are shown in Fig. 1a and b (liver carcinomas and all liver tumors combined, respectively). The male mouse liver tumor data from the two bioassays fall on a consistent dose–response curve indicating no tumor response until absorbed doses exceed approximately 100 mg/kg-d. The female mouse liver tumor data from the two bioassays describe a similarly coherent curve on an absorbed dose basis. However, the curve was shifted with responses occurring at higher doses than in male mice, so only the data for male mice were carried forward for quantitative risk assessment so as to represent the most sensitive response observed.

Taken together, the inhalation bioassay (Aiso et al., 2005) and the gavage bioassay (NTP, 1987) results for male mouse liver tumors show a consistent dose–response curve on an absorbed dose basis. From this analysis it would appear that the mouse liver tumor response is not dependent upon the route of administration and is a result of the total absorbed dose. The consistency in response between the two data sets supports a decision to analyze the combined dataset for dose–response behavior. Because of the similarity in the background tumor incidences, no correction was necessary for this parameter in comparing the tumor responses for the $B6C3F_1$ and BDF_1 mice. Analysis of the combined dataset provides: (1) A better basis for evaluating the shape of the dose–response relationship between an oral dose and tumor response (the oral bioassay is significantly flawed, having only two dose groups, both of which exhibited liver toxicity). (2) Better characterization of the broader dose continuum. (3) An enhanced rationalization of both oral and inhalation

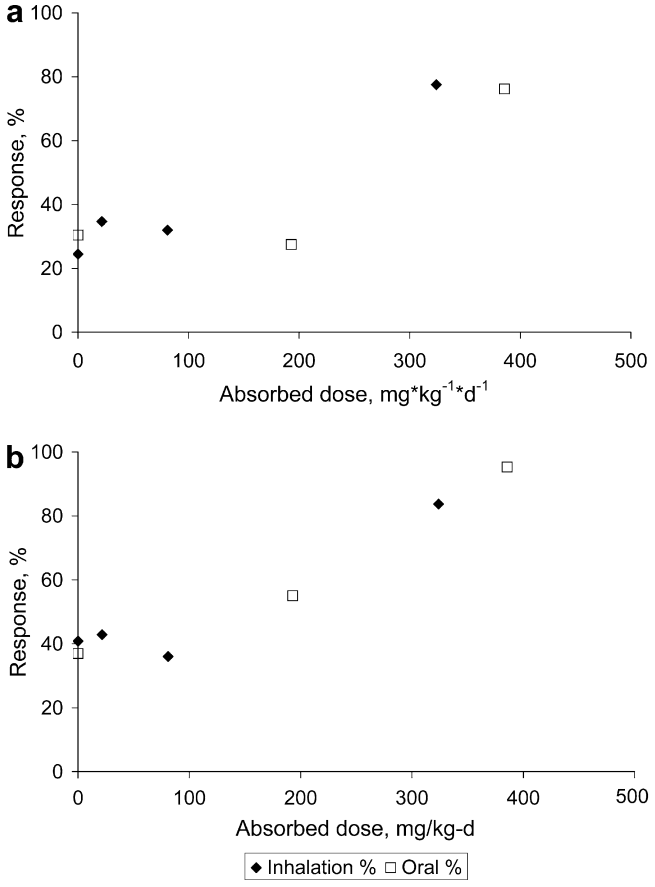

Fig. 1. Liver tumor response data from the gavage bioassay (NTP, 1987) in male $B6C3F_1$ mice and the inhalation bioassay (Aiso et al., 2005) in male BDF_1 mice on an absorbed dose (5 days per week) basis (a, carcinomas only; b, adenomas and carcinomas combined).

exposure risks. The following sections provide a dose–response analysis of the combined tumor dataset to derive a Point of Departure (POD) for 1,4-dichlorobenzene based on the absorbed dose.

12.1. Benchmark dose analysis of the combined data sets

The combined male mouse liver tumor response data set from the two bioassays on an absorbed dose basis was modeled using the EPA Benchmark Dose Software (version 1.3.2). The oral gavage bioassay alone does not allow identification of a benchmark dose for any response rate lower than approximately 50% extra risk because of the lack of a lower, nonresponding dose in the bioassay. However, use of the combined dataset provides sufficient dose–response information to model benchmark responses (BMR) (extra risk) as low as 1% and remain in the range of observation. Benchmark dose modeling was conducted for the combined inhalation and oral gavage data sets for two endpoints: liver carcinomas and combined (adenoma and carcinoma) liver tumors. The modeling for combined liver tumors consistently produced lower benchmark dose estimates compared to those derived based on liver carcinomas only for the same benchmark response levels. Thus,

detailed modeling results are presented here only for the combined liver tumor response endpoint, as this produces the highest estimates of risk.

Table 5 presents the estimated benchmark doses and lower bounds for a 1% response (BMD_{01} and $BMDL_{01}$) for the combined (oral and inhalation) male mouse liver tumor (all liver tumors combined) dataset along with the goodness of fit criteria using the gamma, and Weibull models. The results from these two models provided indistinguishable goodness of fits and the BMD and BMDL estimates were within a factor of two of each other. Without a biological reason for choosing one model over the other, the average BMD and BMDL from the two models will be used throughout the rest of this risk assessment. Additional models from the Benchmark Dose software were evaluated but not used in this risk assessment because of poor fits (results not shown).

The mean BMD_{01} and $BMDL_{01}$ from these two models are 83.7 and 32.6 mg/kg-d (absorbed dose), respectively. The multi-stage model was not used because it provided less robust estimates of the BMDL than the gamma or Weibull models in that it yielded a large disparity (approximately a 15-fold difference) between the estimated BMD and BMDL values. Fig. 2 illustrates the output from the EPA benchmark dose software for the Weibull model, showing that the $BMDL_{01}$ is well within the observed dose range.

Table 5
Results of benchmark dose modeling (1% extra risk) for the combined inhalation and oral gavage bioassay data for male mouse liver tumors (all tumors combined) on an absorbed dose basis

Model	BMR	BMD (mg/kg-d)	BMDL (mg/kg-d)	AIC[a]	p[b]
Gamma	1%	96.8	39.4	380.9	0.91
Weibull	1%	70.6	25.8	380.8	0.92
Averages:		83.7	32.6		

[a] AIC, akaike information criterion.
[b] p-value for fit.

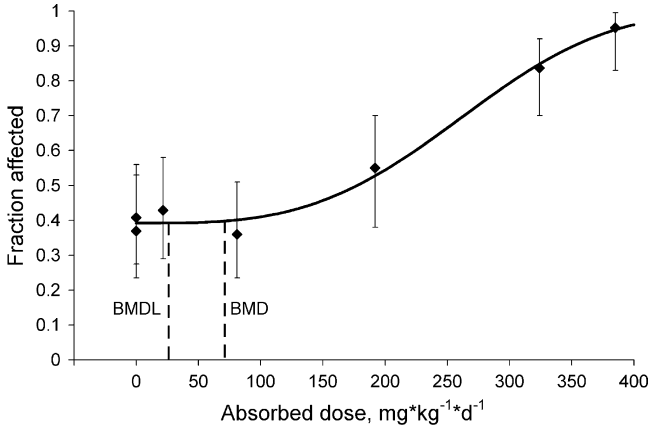

Fig. 2. Output from U.S. EPA BMDS (Weibull model) for the combined data set for combined liver adenomas and carcinomas from the gavage (NTP, 1987) and inhalation (Aiso et al., 2005) bioassays on an absorbed dose basis. The modeled benchmark dose and lower bound are displayed for 1% extra risk. Diamonds are the actual tumor incidences.

This estimate of the $BMDL_{01}$ based on the combined data set can be used as a point of departure for a threshold-based risk assessment or for estimating both oral and inhalation slope factors. The 1% BMR level is justified because it provides a low BMDL that does not require extrapolation beyond the range of observation.

12.2. Nonlinear dose–response (threshold) approach

A nonlinear (threshold) approach is the most scientifically appropriate cancer risk model given the substantial evidence that 1,4-dichlorobenzene induces liver tumors via a nongenotoxic-mitogenic mode action. The combined oral and inhalation bioassay dataset can be used to derive a Point of Departure (POD) by choosing the highest dose group without a statistically significant elevation in tumor response or by choosing a low BMR response level (e.g., 1% extra risk). The highest NOEL in this combined tumor dataset is associated with an absorbed dose of 81 mg/kg-d. From above, the average BMD and BMDL for a 1% response rate are 84 and 33 mg/kg-d, respectively. This range of estimates can be used to characterize a threshold for liver cell mitogenesis and subsequent tumor response.

There is no clear guidance on what an appropriate composite uncertainty factor should be for deriving a dose that would be expected to be free from an elevation in cancer risks. The ideal uncertainty factor (UF) should be sufficiently large to be health protective, without exaggerating the risk such that no actual benefit would be obtained by efforts to comply. The best science indicates that selection of a dose that is protective against the underlying toxicity would be equally protective against the eventual formation of cancer. Thus, a straightforward selection of uncertainty factors similar to those used in protecting against toxicity represent a reasonable approach.

Species differences in metabolism and susceptibility to the endpoint and mechanism of action should help guide the choice of uncertainty factors. The use of data from two chronic bioassays, the clear presence of a NOEL, and the sufficiency of the data for identification of a BMD associated with an estimated 1% response, indicate that no uncertainty factors for either less-than-chronic or LOAEL to NOAEL extrapolation are required.

Based on conventional noncancer reference dose derivation procedures, a factor of 10 would appear reasonable to account for extrapolating from animal to human response. It could well be argued that a factor lower than 10 is justified, given the fact that mice are dramatically more sensitive to spontaneous and induced liver cancer than humans. On the other hand, mitogenic activity is the driving force for cancer induction by 1,4-DCB, and this compound has induced a liver mitogenic response in all species examined thus far including mice, rats, dogs, and guinea pigs, indicating a broad range of activity (U.S. EPA, 2006b). Further, the degree to which precancerous human liver cells might respond to growth stimulation by 1,4-DCB is unknown. These considerations suggest that

Table 6
Estimates for point of departure (POD) and uncertainty factors (UF) and resulting estimates of safe absorbed doses, safe oral intake doses, and safe inhalation exposure concentrations to protect against an increased risk of cancer assuming a nonlinear mode of action

Point of departure		Composite UF	Safe absorbed dose (mg/kg–d)	Safe oral intake dose (mg/kg–d)[b]	Safe inhalation exposure level mg/m3 (ppm)[c]
Description	Value (mg/kg-d)[a]				
NOEL	81	300	0.27	0.3	1.6 (0.3)
BMD_{01}	84	300	0.28	0.3	1.6 (0.3)
$BMDL_{01}$	33	300	0.11	0.1	0.6 (0.1)

[a] Absorbed dose.
[b] Assuming 90% absorption.
[c] Assuming bodyweight of 70 kg, breathing rate of 20 m^3/d, and absorption fraction of 60%.

it is reasonable to keep the species-to-species extrapolation factor at 10. Use of this UF factor eliminates the need to employ a scaling factor to calculate a human equivalent concentration (HEC).

A factor of 10 is commonly used to account for intra-individual (person-to-person) differences in susceptibility. That factor would also appear reasonable in this case.

An additional modifying factor to account for database uncertainties might be added to account for uncertainties in the linkage between mitogenic and carcinogenic activity. The NOAEL, however, is based on actual tumor data and does not rely on measurement of increased liver weight. Nevertheless, the biology is complex and we have added a factor of 3 to account for data deficiencies.

The above considerations yield a composite uncertainty factor of 300. This uncertainty factor of 300 has been applied in the following example to illustrate the range of POD estimates and the resulting safe absorbed dose rates, which range from approximately 0.1 to 0.3 mg/kg-d (Table 6).

Interestingly, the average $BMDL_{01}$ identified in this modeling (33 mg/kg-d) (Table 6) is in the same range as the NOAEL and LOAEL estimates from a key study on liver toxicity in beagle dogs (7 and 36 mg/kg-d, respectively) (Monsanto Company, 1996). As discussed above, tumors resulting from a mitogenic mode of action are likely to occur only at doses that produce notable changes in liver weight and other signs of liver toxicity. The similar values for the point of departure underlying the threshold-based reference value derived above from the actual liver tumor data from the combined oral and inhalation bioassays with two different strains of mice when compared to the point of departure for liver effects in beagle dogs suggests a reasonable degree of species-to-species consistency in susceptibility to 1,4-DCB induced liver toxicity. These observations strengthen the conclusion that exposure levels below those that will cause liver toxicity are likely to be protective of tumorigenic responses, as well as the use of this approach for human risk assessments.

The range of estimates for a safe inhalation exposure level, 0.6–1.6 mg/m^3 (0.1–0.3 ppm) (Table 6), is approximately 8 to 20 times higher than a U.S. EPA proposed RfC of 0.08 mg/m^3 (0.013 ppm) (U.S. EPA, 2006a). That RfC, however, is based on eosinophilic changes to the olfactory epithelium seen in female rats seen at 75 ppm, an endpoint discounted as not representing a toxic lesion by other regulators (European Union, 2004). Safe inhalation exposure levels such as those noted above would be expected to be also protective against an increased risk of cancer.

12.3. Comparison of the mechanism-based vs. the default cancer risk assessment

A default cancer risk assessment would apply a linear extrapolation of risk to zero dose based on the underlying assumption that 1,4-DCB is a genotoxic carcinogen. Interestingly, the External Peer Review Panel for the current draft of the U.S. EPA IRIS risk assessment document has recommended that a cancer risk assessment assuming a nongenotoxic-mitogenic mode of action should be used for 1,4-DCB rather than the default model (U.S. EPA, 2006a, 2006c).

Using the above mechanism-based threshold approach, a representative estimate of an airborne concentration for the human population below which there is unlikely to be any increased risk of cancer during a lifetime is 0.1 ppm (i.e., zero increased risk below that concentration). An assessment based on a default model assuming a genotoxic mode of action and the inhalation bioassay data alone results in an estimate of one in one million increased lifetime risk of cancer at an airborne concentration of approximately 0.00004 ppm (U.S. EPA, 2006a), some 2500-fold lower than the mechanism-based model and 1,875,000-fold lower than the no observed effect concentration for induced cancer of 75 ppm in the inhalation bioassay. These differences in estimates of safe exposure levels are substantial and important from a policy and risk management perspective.

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